ORIGINAL ARTICLE

Phytochemical and pharmacognostical studies of *Blumea lacera* (Roxb.) DC.

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Abstract

Background: *Blumea lacera* (Roxb.) DC belongs to the family Asteraceae which is known to have anthelmintic, diuretics, antipyretic, and many other bioactivities. B. lacera has good medicinal value. Aim: The present work aims to perform a comprehensive phytochemical and pharmacognostical study of *Blumea lacera* (Roxb.) DC. **Materials and Methods:** The Pharmacognostical studies on *Blumea lacera* including parameters such as foreign organic matter, morphological evaluation, Total ash value, acid soluble, water-soluble ash values. Foaming index, Thin layer chromatography (TLC), HPTLC, physico-chemical, analysis and phytochemical studies are established. **Result and Conclusion:** Foreign organic matter of crude drug was found 0.28% w/w. Total ash values of drug was found to be 16.240 and w/w with respect to air-dried crude drug. Moisture content of crude drug was found to be 4.07 % Foaming index of *Blumea lacera* was FI 150. HPTLC studies were carried for the better isolation and Identification of the different components of ethanolic extract of *Blumea lacera*. HPTLC carried out in the solvent system - Toluene: Ethyl acetate: Methanol: Acetic acid (8:1:0.5:0.5). The report of HPTLC indicates the presence of fourteen spots. The results of the study can serve as a valuable resource of pharmacognostic and phytochemical information. Phytochemical characterization of plant extracts revealed the presence of alkaloids, steroids, terpenoids and cardiac glycosides, tannins and phenolic compounds.

Key words: *Blumea Lacera* (Roxb.) DC, high-performance thin-layer chromatography, phytochemical characterization, thin-layer chromatography

The use of natural products or natural product-based medicine is increasing all over the world, especially in the developing countries, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. Approximately 25% of the prescribed drugs in the world are of basically plant origin.[1] In the developing countries like India, approximately 80% people rely on traditional plant-based drugs for their primary health-care needs.[2]

Recent widespread interest in plant-derived drugs reflects its recognition of the validity of many traditional claims regarding the values of natural products in health care.[3] For quality control of traditional medicines, phytochemical investigations are mainly applied. Thus, it makes a great significance to investigate chemical constituents and study pharmacological activity on this plant for its medicinal uses, which will be very useful in the field of medicine as new emerging drug.[4] According to the WHO, medicinal plants are the best sources to obtain a variety of new herbal drug.[5]

Blumea lacera DC. (Asteraceae) is a genus of flowering plants widely distributed in Western and Southern plains of India ascending to 2000 ft in the Himalayas.[6] *B. lacera* is a small annual herb with variable leaves and yellow heads, camphor-like smell. Stem is erect, ash-colored, densely glandular, pubescent. Leaves are often incised. Many flowers arranged in auxiliary cymes or terminal panicle. *B. lacera* also possess these medicinal properties such as sores and wound healing[7] and treating bronchitis.[8] The plant also exhibited antileukemic, antiviral diuretic, styptic,

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astringent, and ophthalmic properties.[9,10] The essential oil from leaves has analgesic, hypothermic, and tranquilizing activities and cytotoxic activities against breast cancer cells and healing cuts.[11] The plant also exhibited stimulant, digestive, anthelmintic, liver tonic, expectorant, febrifuge, anti-inflammatory,[12] antipyretic,[13] and antifungal activities.[14] *B. lacera* (Roxb.) DC. Plant image is shown in Figure 1.

Plant Taxonomy[15]
Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Asterales
Family: Asteraceae
Genus: *Blumea*
Species: *Blumea lacera.*

Vernacular Name/Plant Name in Different Languages[16]

English: Blumea, Malay Blumea
Hindi: Kukaraundha
Gujarati: Kolhar
Telgu: Advimulangi, Karupogaku
Tamil: Kattumullangi, Narakkandai
Bengali: Kukurmuta
Marathi: Bhamurda
Malayalam: Kukkravriksham and Rakkila.

MATERIALS AND METHODS

Plant Material

The fresh whole plant *B. lacera* (Roxb.) DC. were collected during the month of January 2013 from Jaunpur, Uttar Pradesh, India. The plant material was identified and authenticated by Dr. A. K. S. Rawat, Scientist F and Head, National Botanical Research Institute (NBRI), Lucknow (Uttar Pradesh). With reference number NBRI/CIF/368/2013.

The fresh whole plant of *B. lacera* rinsed with tap water followed by distilled water to remove the dirt on the surface. Plant parts were cut into small pieces and shade dried at room temperature then subjected to physical evaluation with different parameters. Dried samples were to ground into fine size powder separately, in a mechanical grinder and then passed through sieve no. 40 to get desired particle size and kept in desiccators until extracted. Then, uniform powder was subjected to standardization with different parameters.

Procedure for Different Parameters

Organoleptic/morphological evaluation

The morphological or organoleptic characters mean to the study of external appearance of herbal materials. Morphological evaluation identifies the degree of purity of such crude drugs. Morphological evaluation is based on shape, size, color, surface characteristics, texture, fracture characteristics, and appearance of the cut surface which are useful for the identification of herbal drugs. The organoleptic evaluation means conclusion reveal characteristics drawn from impressions. The present study of morphological evaluation of crude drug used leaves of *B. lacera* (Roxb.) DC.

Loss on drying (LOD) (gravimetric determination)

The determination of Loss on drying which are based on the loss of material after drying and it is determined by LOD techniques. If there moisture present in crude drugs then which are not observed by visually which encourage crude drugs by microbial growth, the presence of fungi or insects, deterioration and also hydrolysis of crude drugs.. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of moisture. If there is moisture/water present in crude drugs then, which are not observed by visually. Therefore, determination of LOD of crude drug is required to use any technique to remove the moisture as soon as possible.

About 5 g accurately coarse powder of *B. lacera* was taken and transferred to a porcelain dish. After taking the weight of drug, the porcelain dish is kept in a hot air oven for 2 h, during this time, the temperature 100-105°C was maintained. Then, the porcelain dish was kept for cooling in a desiccator at 30-35°C temperature, after cooling the powder was taken and weighed. We have calculated the percentage of LOD with the help of following formula: [17]

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

Determination of ash values

The estimation of ash values of herbal drugs are very important parameter for the determination of quality and purity of herbal drug. In general, the herbal drugs which are available in form of the powder. The main aim of ashing organized drugs is to remove all traces of organic matter which can otherwise interfere in an estimation analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium Ca and Mg.[17]

Determination of total ash value

For the determination of total ash. We have place 2 g of the ground air-dried material, accurately weighed and which are transferred in silica crucible. Spread the material in an

even layer and ignite it by gradually increasing the heat to 500–600°C for 4 h until it is white, indicating the absence of carbon. Cool in a desiccator and weigh. We have calculated the percentage of total ash value as per the reference of the air-dried crude drug with the help of following formula (Indian Pharmacopoeia, 1996):[18]

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken ash}} \times 100$$

Acid insoluble ash

The total ash obtained as above was cover with a watch glass boiled gently with 25 ml of hydrochloric acid for 5 min.Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. The insoluble matter was collected in an ashless filter-paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hotplate, and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 min, then weighed without delay. Calculate the content of acid-insoluble ash in mg/g of air-dried material.

Water soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 min. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Wash with hot water and ignite in a crucible for 15 min at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg/g of air-dried material.

Foaming index

To take accurately weight 1 g of powder crude drug and transfer in 500 ml of flask which contains 100 ml of boiling water. The temperature is maintained and the drug boils continuously for 30 min. When the solution is cool, then the solution was filtered it into a volumetric flask and make a volume addition of water up to 100 ml. Take the above solution into ten graduated test tubes and each test tube containing 1ml, 2 ml, 3 ml up to 10 ml, respectively. Then, we have adjusted the volumes up to 10 ml in each test tube. The test tubes are cover and shake for 15 s. After shaking, the test tubes are kept aside for 15 min and measure the foam height.[19]

The results assessed as follows:

I. The each and every test tube having the foaming index is less than 100 when the foam height is 1 cm.
II. If a height of foam of 1 cm is measured in any tube, the volume of the herbal material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
III. When the foam height observed above one centimeter in each and every test tube. Then, there is a need to the determination of the 1000 foaming index. Take one or more tests using the different decoction dilutions and find out the result.

Where a = Volume of the decoction in 100 ml which are preparing from the dilutions in the test tube where height of foaming is observed.

$$\text{Foaming index} = \frac{100}{a}$$

Determination of foreign organic matter

During storage, products should be kept in a clean and hygienic place, so that no contamination occurs. Special care should be taken to avoid the formation of molds since they may produce aflatoxins. Any soil, stones, sand, dust, and other foreign inorganic matter must be removed before herbal materials are cut or ground for testing.

About 100 g of the sample was weighed and spread out in a thin layer on the paper. The foreign matter was detected by inspection with the use of a lens (6×). It was then separated and weighed and the percentage of foreign organic matter was calculated.[19]

Preparation of ethanolic and petroleum ether extracts

An about 50 g powder of *B. lacera* was subjected to extraction. The extraction was carried out in a Soxhlet apparatus for 10 h using petroleum ether (40–60ºC) and ethanol (95%). The extraction was continued until the solvent in the thimble became clear. After the effective extraction, the solvent was then evaporated using rotary evaporator and the extract obtained with each solvent was weighed. Its percentage was calculated and the color and consistency of the extract were noted. These extracts were stored in a desiccator. These obtained extracts were subjected to chemical investigation. The results were recorded in Table 1.[17]

Phytochemical screening

The phytochemical screening was performed crude extract of (petroleum ether and ethanolic extract) of *B. lacera*. One gram of the ethanol extract of *B. lacera* was dissolved in 100 ml of ethanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents present.

Photochemical tests were done in plant extracts for the detection of the presence of different chemical constituents such as alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins, and other substances which are responsible for the biological activity. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals for the detection of different chemical constituents are observed.[17]

Thin-layer chromatography (TLC)

TLC is the method mainly uses to investigate the presence of chemical constituent qualitatively and quantitative in the

plant extract. It is used to investigate alkaloids, glycosides, triterpenoids, lipid component, sugar, and their derivates, etc. This component can also run with standards for the investigation. It is an easy, versatile, and reliable method to establish authenticity, identity, and purity.

"Their relative polarities which related to the type and number of functional groups present on a molecule capable of hydrogen bonding"

$$R_f = \frac{\text{Distance travelled by solute front from origin line}}{\text{Distance travelled by solvent front from origin line}}$$

Where R_f = Retention factor

The ethanolic extract of *B. lacera* was subjected to TLC studies, to find the presence of number of compounds which support by the chemical test.

Thin layer chromatography of Blumea lacera was performed in different solvent system like, Hexane: ethyl acetate: Methanol (10: 3:1.5), Chloroform: Benzene: Acetic acid (3:1:1) show three spot, Chloroform: Benzene: Acetic acid (3:1:0.5) show five spots.

High-performance TLC (HPTLC)

Standardized manufacturing procedures and suitable analytical tools are required to establish the necessary framework for quality control in herbals. Among those tools, separation techniques including high-performance liquid chromatography, HPTLC, and capillary electrophoresis are the most widely used to establish reference fingerprints of herbs, against which raw materials can be evaluated and finished products can be assayed.

HPTLC also known under the synonym planar chromatography which is a modern, powerful analytical technique with is separation power and reproducibility superior to TLC.

HPTLC Parameter

Sample preparation: 10 mg/ml
Sample application: Linomat 5 applicator (Camag)
Volume applied: 10 µl
Solvent system: Toluene:ethyl acetate:methanol:acetic acid (8:1:0.5:0.5)
TLC plate development: Presaturated Camag Twin Trough Chamber.

RESULTS AND DISCUSSION

Morphological Evaluation

Herbal materials are categorized according to sensory, macroscopic, and microscopic characteristics. An examination to determine these characteristics is the first step toward establishing the identity and the degree of purity of such materials and should be carried out before any further tests are undertaken. The present study of morphological evaluation of crude drug used leaves of *B. lacera* (Roxb.) DC. The observation of organoleptic characteristics which are shown in Table 2.

Figure 1: *Blumea lacera* (Roxb.) DC.

Table 1: The characteristics of ethanolic extract of *B. lacera* (Roxb.) DC

Characteristics	Ethanolic extract
Extractive value	2.76
Physical appearance	Semi-solid
Color	Dark brown
Odor	Unpleasant
Taste	Bitter

B. lacera: Blumea lacera

Table 2: Macroscopic characteristics of *B. lacera* (Roxb.) DC. leaves

***B. lacera* (Roxb.) DC**	
Color	**Pale green**
Odor	Strong odor of turpentine
Taste	Bitter
Size and shape	Leaves alternate, simple, or shortly petiole

B. lacera: Blumea lacera

LOD

LOD of crude drug an excess of water in herbal materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content should, therefore, be set for every given herbal material. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. We have calculated the percentage of LOD. The

result of LOD of drug is shown in Table 3. Moisture content of *B. lacera* was found to be 4.07.

Estimation of ash values

The ash remaining following ignition of herbal materials is determined by three different methods which measure total ash, acid-insoluble ash, and water-soluble ash. We have calculated of total ash value of *B. lacera* with the help of following formula which is shown in Table 4. Total ash values of *B. lacera* were found to be 16.240 w/w with respect to air-dried crude drug. The percentage of acid insoluble ash was calculated with reference to the air-dried. The results were recorded in Table 5.

Total acid insoluble ash values of *B. lacera* were found to be 0.780 w/w with respect to air-dried crude drug. The percentage of water-soluble ash was calculated with reference to the air-dried drug. The results were recorded in Table 6.

Water-soluble ash of *B. lacera* was found to be 6.857% w/w, respectively, with respect to air-dried crude drug.

Foaming index

Many herbal materials contain saponin that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index.

Table 3: Total ash of *Blumea lacera*

S. No.	Weight of empty Crucible (g)	Weight of crucible + drug (before ignition) (g)	Weight of drug Taken (g)	Weight of crucible after ignition (g)	Weight of total ash (g)
1.	16.325	18,347	2,022	16.654	0.329
2.	16.994	19.015	2,021	17.318	0.324
3.	16.496	18.517	2.021	16.820	0.324
4.	Mean				0.325

Results are expressed as Mean n = 3

Table 4: Acid insoluble ash of *Blumea lacera*

S. No.	Weight of drug taken (g)	Weight of empty crucible (g)	Weight of crucible + acid insoluble ash (g)	Weight of Acid insoluble ash (g)	Acid insoluble ash (% w/w)
1.	2.024	16.325	16.342	0. 017	0.849
2.	2.021	16.993	17.007	0.014	0.692
3.	2.021	16.495	16.511	0. 016	0.791
Mean					0.777

Results are expressed as Mean n = 3

Table 5: Water Soluble Ash of *Blumea lacera*

S. No.	Weight of drug taken (g)	Weight of empty Crucible (g)	Weight of Water insoluble ash (g)	Water Soluble ash (%w/w)
1.	2.024	16,326	0.161	8.181
2.	2.021	16.998	0.158	7.817
3.	2.021	16.496	0.154	7.619
Mean				7.855

Results are expressed as Mean n = 3

Table 6: Water soluble ash of *B. lacera*

Weight of empty crucible (g)	Weight of water-insoluble ash (g)	Weight of total ash (g)	Water soluble ash(%w/w)
16,326	0.988	9.179	8.181
16.998	1.296	9.227	7.924
16.489	1.340	8.804	7.460
Mean			

B. lacera: Blumea lacera

The results were recorded in Table 7. Foaming index of *B. lacera* is FI 150

Determination of foreign organic matter

Herbal materials should be entirely free from visible signs of contamination by molds or insects, and other animal contamination, including animal excreta. The results were recorded in Table 8. Foreign organic matter of crude drug was found to be >1% w/w.

Phytochemical Screening

The phytochemical screening was performed crude extract of (petroleum ether and ethanolic extract) of *B. lacera*. Phytochemical Screening of plant extracts petroleum ether and ethanolic extracts of Blumea lacera revealed the presence of alkaloids, steroids, terpenoids and cardiac glycosides, tannins and phenolic compounds. The result of Phytochemical Screening was recorded in Table 9.. The qualitative results

Table 7: Foaming index of *B. lacera*

Dilutions (drug extract+water)	Height of foam in standard (mm)	Height of foam in leaves decoction (mm)
1:9	1	<10
2:8	2	<10
3:7	3	<10
4:6	6	<10
5:5	6	<10
6:4	8	<10
7:3	8	<10
8:2	9	<10
9:1	10	<10
10:0	10	<10

B. lacera: Blumea lacera

Table 8: Determination of foreign organic matter of *B. lacera*

Weight of crude drug taken (g)	Weight of drug after removal of foreign matter (g)	Weight of foreign matter (g)	Foreign matter (%w/w)
100	99.680	0.320	0.32
100	99.725	0.275	0.27
100	99.740	0.260	0.26
Mean			0.28

B. lacera: Blumea lacera

Figure 2: Thin layer chromatography of ethanolic extract of *Blumea lacera*

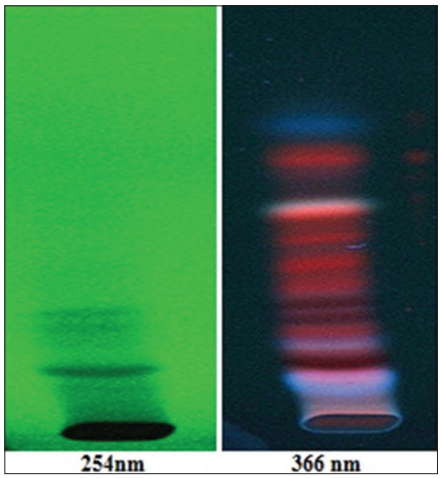

Figure 3: High Performance Thin Layer Chromatography of ethanolic extract of *Blumea lacera*

Table 9: Phytochemical analysis of *B. lacera*

Chemical test	Petroleum extract	Ethanolic extract
Test for alkaloids		
Mayer's reagent	+	+
Dragendorff's reagent	+	+
Wagner reagent	-	+
Hager's test		
Test for Carbohydrates		
Molisch's test	-	-
Fehling's test	-	-
Test for Glycosides		
Keller–Kiliani test	+	+
Sodium Nitrosopruside test	-	-
Borntrager's test	+	+
Test for phenolic compounds and tannins		
Ferric chloride test	-	+
Lead acetate test	-	+
Test for flavonoids		
Ammonia test	+	+
Pew test for flavonoids	-	+
Test for Proteins and free Amino Acids		
Million's test	-	+
Ninhydrin test	+	+
Biuret test	-	-
Xanthoprotein test	-	-
Test for Steroids		
Salkowski test	+	+
Liebermann–Burchard test	-	+
Test for triterpene	+	+
Test for fats and oils	+	-

B. lacera: Blumea lacera, + = Present, - = Absent

are expressed as (+) for the presence and (-) for the absence of phytochemicals for the detection of different chemical constituents are observed in Table 9.

TLC

Thin-layer chromatography is particularly valuable for the qualitative determination of small amounts of constituents. Thin-layer chromatography is effective and easy to perform, and the equipment required is inexpensive, the technique is frequently used for evaluating herbal materials and their preparations. TLC studies of *B. lacera* was performed in different solvent system. Toluene: ethyl acetate:methanol: acetic acid (8:1:0.5:0.5) solvent system show seven spots. Visualizing agent is vanillin iodine and anisaldehyde. These TLC spots with R_f value and color are shown in Table 10 and TLC plate is shown in Figure 2.

Table 10: TLC of ethanolic extract of *B. lacera*

R_f values	Colour
0.15	Brown
0.27	Brown
0.32	Green
0.40	Yellow
0.70	Brown
0.82	Yellow
0.95	Yellow

TLC: Thin layer chromatography, *B. lacera: Blumea lacera*

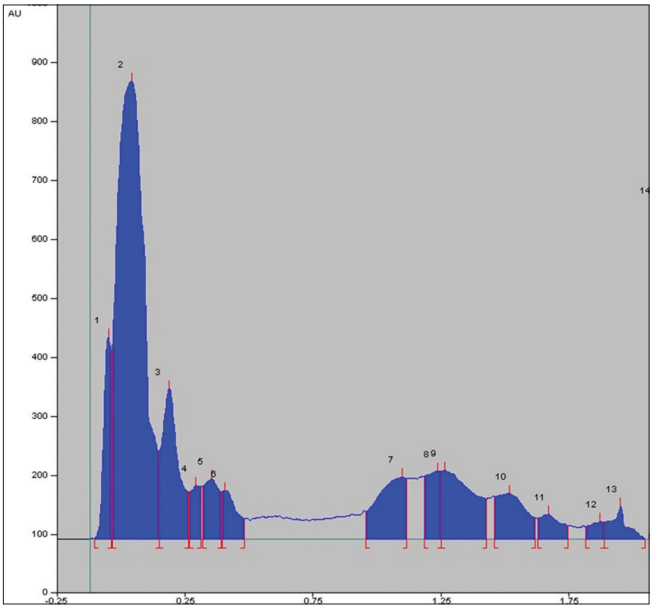

Figure 4: High Performance Thin Layer Chromatography finger printing of ethanolic extract of *Blumea lacera*

HPTLC

HPTLC studies were carried for the better isolation and identification of the different components of ethanolic extract of *B. lacera*. The report of HPTLC indicates the presence of 14 spots in the solvent system - Toluene:ethyl acetate:methanol:acetic acid (8:1:0.5:0.5) carried out at NBRI, Lucknow. These HPTLC spots with R_f value and color are shown in Table 11, and HPTLC plate is shown in Figure 3 and Figure 4 is given respectively.

Table 11: *HPTLC of ethanolic* extract of *B. lacera*

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %	Assigned substance
1.	−0.11Rf	0.6AU	−0.05Rf	343.3AU	12.44	0.04Rf	19.2AU	4602.4AU	5.49	Unknown
2.	−0.04Rf	324.9AU	−0.04Rf	777.2AU	28.16	0.15Rf	48.4AU	37710.2AU	44.94	Unknown
3.	0.15Rf	149.1AU	0.19Rf	255.3AU	9.25	0.26Rf	79.9AU	7414.2AU	8.84	Unknown
4.	0.27Rf	80.8AU	0.29Rf	91.0AU	3.30	0.31Rf	39.6AU	1565.6AU	1.87	Unknown
5.	0. 32Rf	89.8AU	0. 35Rf	102.7AU	3.72	0.39 Rf	30.6AU	2881.1AU	3.43	Unknown
6.	0.40Rf	80.7AU	0.41Rf	82.1AU	2.97	0.48Rf	35.1AU	2105.7AU	2.51	Unknown
7.	0.96Rf	46.6AU	1.10 Rf	105.8AU	3.83	1.12Rf	02.8AU	5356.0AU	6.38	Unknown
8.	1.19Rf	106.1AU	1.24 Rf	115.9AU	4.20	1.25Rf	15.9AU	3020.4AU	3.60	Unknown
9.	1.25Rf	115.8AU	1.26 Rf	117.1AU	4.24	1.43Rf	38.3AU	6553.0AU	7.81	Unknown
10.	1.46Rf	72.0AU	1.52Rf	77.9AU	2.82	1.62Rf	35.7AU	4101.8AU	4.89	Unknown
11.	1.63Rf	35.3AU	1.67Rf	42.4AU	1.53	1.57Rf	23.0AU	1635.5AU	1.95	Unknown
12.	1.81Rf	21.3AU	1.87Rf	30.5AU	1.11	1.89Rf	29.2AU	785.6AU	0.94	Unknown
13.	1.89Rf	29.1AU	1.95Rf	55.8AU	2.02	2.05Rf	0.5AU	1512.7AU	1.80	Unknown
14.	2.09Rf	20.1AU	2.08Rf	563.0AU	20.40	2.10Rf	52.1AU	4660.0AU	5.5	Unknown

HPTLC: High-performance thin layer chromatography, *B. lacera: Blumea lacera*

CONCLUSION

It can be concluded that the from above the study results of *Blumea lacera* can serve as a valuable resource of pharmacognostic and phytochemical information. Phytochemical characterization of plant extracts revealed the presence of alkaloids, steroids, terpenoids, cardiac glycosides, tannins and phenolic compounds present in this plant. Further studies are going on this plant to isolate, identify, characterize, and elucidate the structure of the bioactive compounds.

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